ESC European Society of Cardiology

Cardiovascular Research (2022) **00**, 1–11
https://doi.org/10.1093/cvr/cvac014

REVIEW

The role of the Hippo pathway in autophagy in the heart

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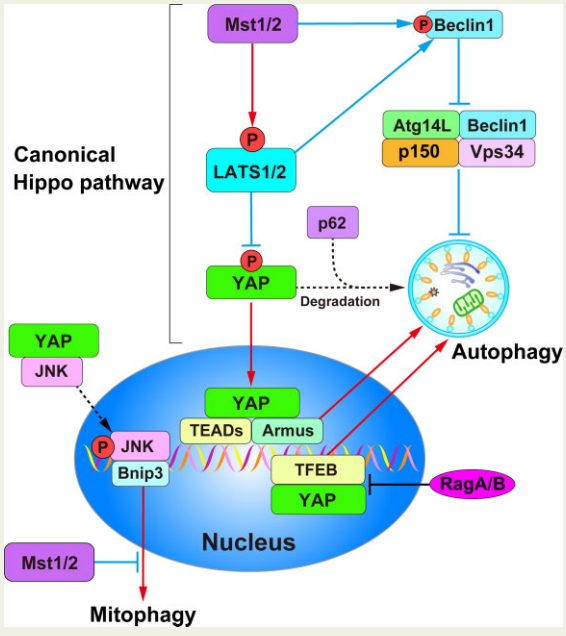
Received 30 October 2021; editorial decision 21 January 2022; accepted 7 February 2022; online publish-ahead-of-print 12 February 2022

Abstract

The Hippo pathway, an evolutionarily conserved signalling mechanism, controls organ size and tumourigenesis. Increasing lines of evidence suggest that autophagy, an important mechanism of lysosome-mediated cellular degradation, is regulated by the Hippo pathway, which thereby profoundly affects cell growth and death responses in various cell types. In the heart, Mst1, an upstream component of the Hippo pathway, not only induces apoptosis but also inhibits autophagy through phosphorylation of Beclin 1. YAP/TAZ, transcription factor co-factors and the terminal effectors of the Hippo pathway, affect autophagy through transcriptional activation of TFEB, a master regulator of autophagy and lysosomal biogenesis. The cellular abundance of YAP is negatively regulated by autophagy and suppression of autophagy induces accumulation of YAP, which, in turn, acts as a feedback mechanism to induce autophagosome formation. Thus, the Hippo pathway and autophagy regulate each other, thereby profoundly affecting cardiomyocyte survival and death. This review discusses the interaction between the Hippo pathway and autophagy and its functional significance during stress conditions in the heart and the cardiomyocytes therein.

Graphical Abstract



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Keywords Hippo pathway • Autophagy • Mst1 • YAP • Heart

1. Introduction

The Hippo pathway regulates a variety of biological processes, including cell growth and death, organ size control, and tissue regeneration.[1] This evolutionarily conserved signalling cascade was first discovered through genetic screening in *Drosophila melanogaster* to identify gene mutations causing tissue overgrowth.[2] This work identified the core components of the Hippo pathway, including the Ste20-like protein kinase Hippo (Hpo), the WW domain-containing protein Salvador (Sav), the nuclear Dbf2-related (NDR) family protein kinase Warts (Wts), the adaptor protein Mob as tumour suppressor (Mats), and the transcriptional co-activator Yorkie (Yki).[3–5] In addition, the Scalloped (Sd) family transcription factors were also identified as nuclear effectors of the Hippo signalling cascade,[6] and a signal from Fat, a proto-cadherin, was shown to suppress tissue growth in *Drosophila* via Hippo-mediated signalling.[7] Subsequent studies demonstrated that the Hippo pathway is conserved from *Drosophila* to mammals.[1] Increasing evidence suggests that the Hippo pathway is also involved in a wide variety of cellular functions besides growth and death, including epigenetics, metabolism, mitochondrial function, and autophagy.

Autophagy is an evolutionarily conserved mechanism of lysosome-mediated cellular degradation.[8] It is activated in response to energy shortage and, through lysosomal degradation, recycles amino acids and fatty acids to regenerate ATP, proteins, and organelles. Autophagy also plays an essential role in mediating cellular protein and organelle quality control mechanisms. Thus, autophagy is essential for the maintenance of cellular homeostasis during stress. Conversely, dysregulated activation of autophagy leads to cellular malfunction and death caused by either excessive degradation or excessive intracellular accumulation of autophagosomes.[9]

Growing evidence suggests that autophagic activity is altered in response to changes in Hippo pathway activity, which, in turn, positively or negatively affects growth and death in many cell types.[10] On the other hand, the activity of the Hippo pathway is also affected by autophagy, such that autophagy indirectly controls various cellular functions through the Hippo pathway.[11] This interaction between the Hippo pathway and autophagy is observed in the heart and the cardiomyocytes therein, with profound effects upon cardiomyocyte survival and death.[12,13] In this review, we discuss the current understanding of the regulation of autophagy by the Hippo pathway in cardiomyocytes and the functional significance of the Hippo–autophagy interaction in various stress conditions in the heart.

2. The Hippo pathway in mammals

The Hippo pathway largely consists of upstream kinases, which promote cell death and inhibit cell growth, and nuclear transcription factor co-factors, which inhibit cell death and promote cell growth (*Figure 1*). The upstream kinases negatively affect the nuclear factors, namely yes-associated protein (YAP; Yki orthologue) and transcriptional co-activator with PDZ-binding motif (TAZ), by inducing phosphorylation and nuclear exit or degradation. Mammalian orthologues of Hpo, one of the major components of the Hippo pathway, are mammalian sterile 20-like protein kinase 1 and 2 (Mst1/2). Mst1/2 form heterodimers with Salvador family WW domain-containing protein 1 (Sav1; Sav orthologue) through their carboxyl-terminal SARAH domains.[14] The interaction between Mst1/2 and Sav1 is essential for phosphorylation of Sav1, MOB kinase activator 1 (MOB1A; Mats orthologue), and large tumour suppressor kinase 1/2 (LATS1/2; Wts orthologues) by Mst1/2.[15,16] On the other hand, both Mst1 and LATS2 are negatively regulated by neddylation in cardiomyocytes.[17] Activated LATS1/2 phosphorylate YAP and TAZ at multiple sites. Phosphorylation of YAP at serine (Ser) 127 promotes its binding to 14-3-3 and cytoplasmic retention, while phosphorylation at Ser381 triggers its degradation through the SCF-β-TRCP-

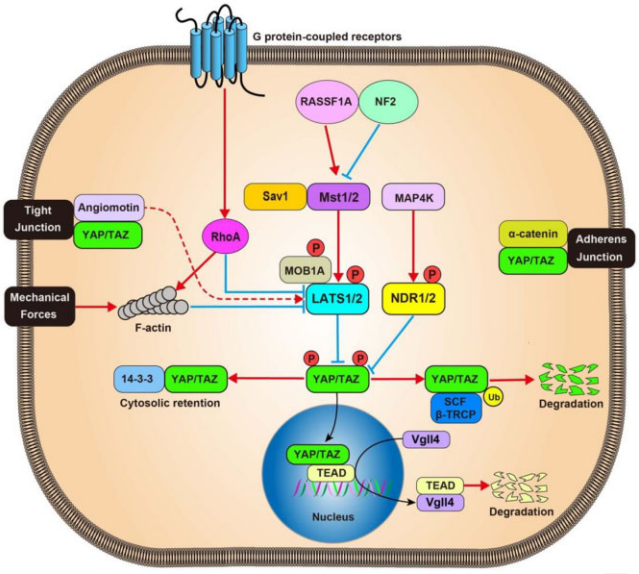

Figure 1 A schematic representation of Hippo signalling in mammals. Core components of the mammalian Hippo pathway comprise Mst1/2, Sav1, MOB1A, and LATS1/2. This pathway is modulated by various stimuli, including G protein-coupled receptor signalling, mechanical forces and cell–cell interactions. The upstream kinases negatively regulate YAP/TAZ via phosphorylation, thereby promoting their degradation and/or protein–protein interactions that favour cytosolic retention of YAP/TAZ (see text for details and definitions of abbreviations). Red lines indicate facilitatory pathways, and blue lines indicate repressive ones. β-TRCP, β-transducin repeat containing protein; LATS1/2, large tumour suppressor kinase 1 and 2; MOB1A, MOB kinase activator 1; Mst1/2, mammalian sterile 20-like protein kinase 1 and 2; NDR, nuclear Dbf2-related kinase family; NF2, Neurofibromin 2; P, phosphorylation; RASSF1A, Ras-association domain family isoform 1 A; Sav1, Salvador family WW domain-containing protein 1; SCF, skp1, cul1, and F-box protein; TAZ, transcriptional co-activator with PDZ-binding motif; TEAD, transcriptional enhanced associate domain; Ub, ubiquitination; Vgll4, vestigial like family member 4; YAP, Yes-associated protein.

mediated ubiquitination system.[18,19] Furthermore, phosphorylation of YAP at Ser94 by AMPK prevents YAP association with the TEA domain (TEADs; Sd orthologues) transcription factors.[20] TAZ is similarly phosphorylated by LATS1/2.[21] Phosphorylation of YAP/TAZ at these sites inhibits the transcriptional activity of YAP/TAZ downstream targets, including TEAD family members 1–4, FoxO1, Myb, RUNX1/2, Tbx5, ErbB4, SMADs, HIF-1α, and TFEB (reviewed in Reference[22]) thereby regulating a wide variety of functions in cells. Vgll4, a TEAD1 binding protein, also inhibits YAP–TEAD interaction when it is deacetylated, thereby causing degradation of TEAD1 and inhibiting postnatal cardiomyocyte proliferation.[23] In addition, YAP regulates IGF signalling,[24] the Wnt pathway,[25] gp130,[26] PI3Kcb,[23] the Akt pathway,[27] and miR-206[28] through unknown mechanisms, thereby regulating cell growth responses in cardiomyocytes.

Recently, novel members of the core cassette of the Hippo pathway, the Ste20-like MAP4K family and the NDR kinase family (NDR1 and NDR2), have been identified.[29] Mechanistically, MAP4K family kinases are responsible for activating phosphorylation of both LATS1/2 and NDR1/2, additional YAP kinases that negatively regulate YAP/TAZ.[30]

The Hippo pathway is regulated by cellular mechano-transduction, G protein-coupled receptor ligands, DNA damage, cell–cell contact, and cell polarity.[1] These mechanisms affect the phosphorylation and nuclear localization of YAP primarily through regulation of upstream kinases, such as Mst1/2 and LATS1/2.[31] In addition, mechanical forces directly induce nuclear entrance of YAP,[32] and other factors, including angiomotin and α-catenin, prevent nuclear localization of YAP through sequestration.[33,34] Mechanical forces can alter substrate or extracellular matrix stiffness, thereby changing the subcellular localization of YAP/TAZ and promoting their activity.[35,36] The actin cytoskeleton mediates not only the transduction of mechanical forces but also Hippo pathway activity. F-actin, a major component of actin filaments, positively regulates YAP activity by promoting LATS1/2 phosphorylation.[37] RhoA activation in response to G protein-coupled receptor-mediated stimuli promotes LATS1/2 phosphorylation, as well as stress fibre formation and actin cytoskeleton rearrangement, thereby enhancing YAP activity.[38,39] The α-catenin complex, a component of adherens junctions located in the cell membrane, negatively regulates YAP/TAZ activity by interacting with them and suppressing their nuclear localization.[34,40] Similarly, angiomotin, which associates with tight junctions at the cell membrane, also associates with YAP/TAZ, thereby suppressing their activity.[33] In parallel, the angiomotin complex enhances LATS2 activity to inhibit YAP activation.[41] Other factors, including Xinβ,[42] an intercalated disc protein, dystrophin-glycoprotein complex,[43] and IFT88,[44] a protein involved in primary cilia and centrosome, also affect the level of YAP through direct protein–protein interaction in the heart.

In the heart, Mst1/2 and LATS2 are activated by myocardial ischaemia/reperfusion,[45] oxidative stress,[45] high fat diet consumption,[46] and chronic volume[13,47] and pressure overload.[48,49] Stress-induced activation of the upstream Hippo kinases induces apoptosis and inhibits autophagy in cardiomyocytes, thereby detrimentally affecting the heart.[13,45–48,50] Their effects may be mediated through phosphorylation of either YAP/TAZ or other targets, including Bcl-xL,[51] Beclin 1,[13] and mTORC1/2.[52,53] Since the effect of the latter is mediated through a YAP/TAZ-independent mechanism, it is referred to as the non-canonical Hippo pathway. YAP and TAZ are activated during the acute phase of pressure overload through RhoA-dependent mechanisms.[54] Activation of YAP is also observed in the heart during the chronic phase of obesity cardiomyopathy[55] and in a mouse model of lysosomal storage disorder (LSD).[12] On the other hand, YAP and TAZ are down-regulated in the heart during chronic pressure overload[48] and post-myocardial infarction (MI) cardiac remodelling,[27] primarily through activation of the Hippo kinases. Down-regulation of YAP is also observed in some forms of cardiomyopathy.[56] YAP promotes either hypertrophy[28,54] or proliferation of cardiomyocytes,[25,57–62] thereby promoting compensatory hypertrophy or myocardial regeneration. YAP also promotes cardiomyocyte survival by up-regulating anti-oxidant genes via stimulation of FoxO1[63] and Pitx2.[64] Thus, down-regulation of YAP below physiological levels facilitates heart failure. In fact, interventions that stimulate YAP have been shown to improve cardiac function by promoting myocardial regeneration.[25,57,58,64,65] It should be noted, however, that excessive activation of YAP can be detrimental in some cardiac conditions,[66] including chronic pressure overload[48,55] and LSDs.[12] For example, excessive activation of YAP induces de-differentiation of cardiomyocytes and consequent contractile failure.[55,66] Thus, caution should be exercised when considering stimulation of YAP as a therapeutic option for treatment of cardiac conditions. In addition, YAP function is cell-type specific. Increasing lines of evidence suggest that YAP is activated in non-myocytes, including cardiac fibroblasts and macrophages, in response to cardiac stress, thereby promoting fibrosis and inflammation.[67–70] Thus, changes in YAP activity during stress have a significant influence in the heart but the effect is complex due to the cell-type specificity in downstream signalling mechanisms.

3. The autophagy machinery

Autophagy degrades dysfunctional organelles and aggregated proteins in response to various types of cell stress, including starvation, oxidative stress, hypoxia, endoplasmic reticulum (ER) stress, and infection.[71] Four different types of autophagy have been identified: macroautophagy, microautophagy, chaperone-mediated autophagy, and alternative autophagy.[52] Of these, macroautophagy is the most common type, and is commonly referred to simply as autophagy. The initial step of autophagy is formation of an isolation membrane (also called a phagophore). The isolation membrane then elongates to form a double-membrane vesicle, called an autophagosome, that sequesters cytosolic components and delivers them to lysosomes for degradation. The products of autophagic degradation are then exported to the cytoplasm for recycling.[72,73] Autophagy was initially thought to be a non-selective degradation process. However, there is a growing body of evidence that suggests autophagy can specifically eliminate dangerous cytosolic objects, such as dysfunctional organelles (mitophagy, lipophagy, ER-phagy, lysophagy, and nucleophagy), protein aggregates (aggrephagy), and invading pathogens (xenophagy), as well.[73–75] On the other hand, excessive activation of autophagy can cause cell death. Autosis, one form of autophagic cell death, was initially observed in response to Tat-Beclin1, a cell-penetrating autophagy-inducing peptide.[76] It is also observed in the heart during the late phase of reperfusion injury.[77] Autosis has unique morphological characteristics, including nuclear convolution at early stages, increased numbers of autophagosomes/autolysosomes, and focal swelling of the perinuclear space at late stages.[78]

The autophagy machinery is tightly regulated by autophagy-related genes (ATG)-encoded proteins (*Figure* 2). These proteins were initially

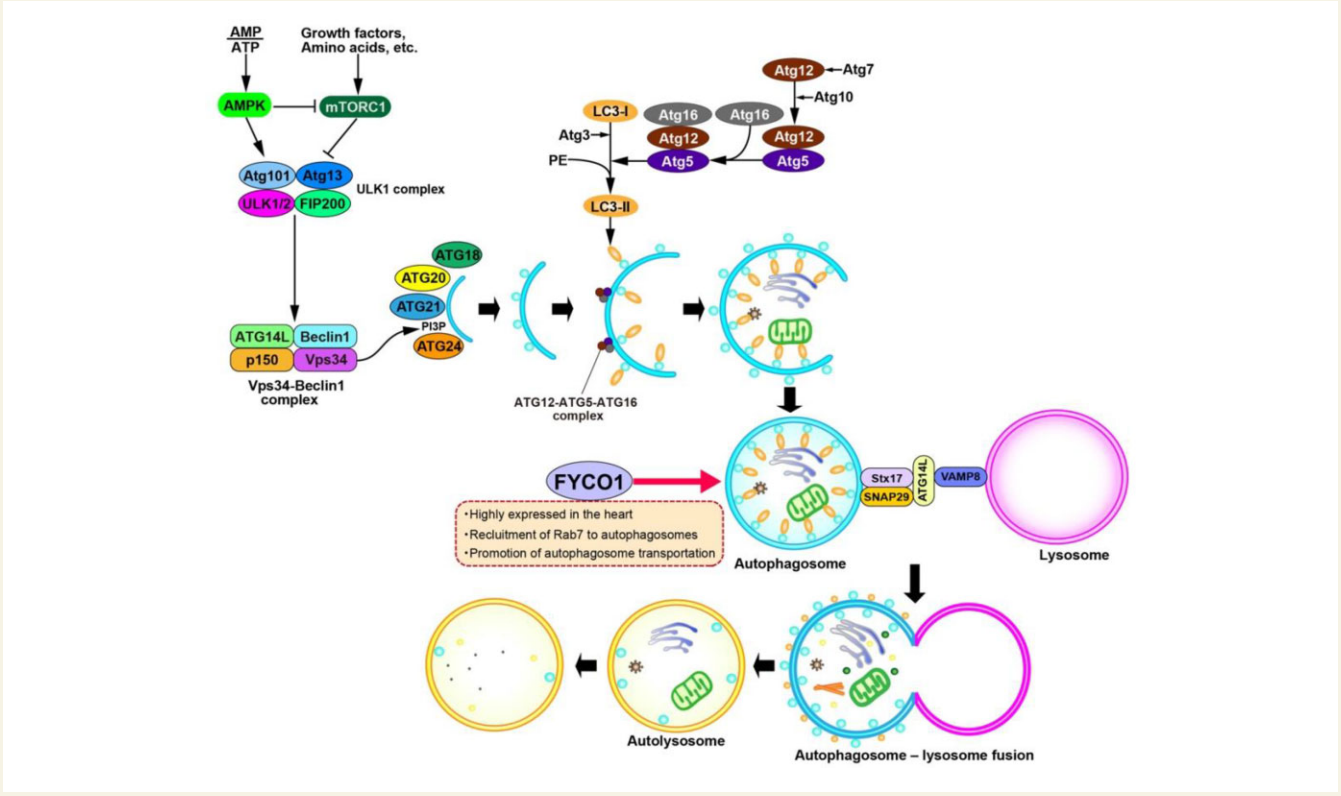

Figure 2 A schematic representation of the autophagy machinery in mammals. The autophagy machinery is tightly regulated by autophagy regulatory proteins, including ATG-encoded proteins (see text for details and definitions of abbreviations). AMPK, AMP-activated protein kinase; FIP200, FAK family interacting protein of 200 kDa; FYCO1, FYVE and coiled-coil domain autophagy adaptor 1; LC3, microtubule-associated protein light chain 3; mTOR, mammalian target of rapamycin; PI3P, phosphatidylinositol 3-phosphate; SNAP29, synaptosomal-associated protein 29; Stx17, Syntaxin 17; ULK1/2, unc-51-like autophagy-activating kinase 1 and 2; VAMP8, vesicle associated membrane protein 8; Vps34, vacuolar protein sorting 34.

discovered in *Saccharomyces cerevisiae* (yeast) in pioneering studies conducted by Ohsumi's group.[79] Thus far, more than 30 ATGs have been identified, most of which are evolutionarily conserved.[80] Nutrient deprivation or a lack of growth factor supplementation triggers phagophore formation mediated through activation of the unc-51 like autophagy-activating kinase (ULK) macromolecular complex, which is composed of ULK1 or ULK2 (mammalian homologues of yeast ATG1), focal adhesion kinase family interacting protein of 200 kD (FIP200), ATG13, and ATG101, thereby inactivating and dissociating mammalian target of rapamycin (mTOR).[81] The activated ULK complex then activates another macromolecular protein complex comprising Vps34, Beclin 1 (mammalian homologue of yeast ATG6), ATG14L, and p150 (mammalian homologue of yeast Vps15).[82,83] Specifically, ULK1 phosphorylates Beclin1 at Ser14, thereby recruiting the Vps34 complex, a class III phosphatidylinositol-3 (PI3)-kinase complex I, to promote PI3-phosphate generation, which results in the translocation of multiple ATGs, including ATG18, ATG20, ATG21, and ATG24, to the phagophore assembly site and growth and expansion of the phagophore.[83–85] The maturation and closure of the autophagosome is carried out by the ATG conjugation system, which resembles the ubiquitin-conjugating system. The ubiquitin-like molecule ATG12 is first activated by ATG7, an E1 like protein, allowing it to conjugate to ATG5 via the E2 ligase-like ATG10 protein.[86,87] Next, the ATG12-ATG5 complex causes phagophore elongation by interacting with ATG16, an E3-like enzyme, to promote lipidation of microtubule-associated protein 1 light chain 3 (LC3; ATG8 family protein) by conjugating it to phosphatidylethanolamine.[88] The conjugated form of LC3, LC3-II, is then integrated into the phagophore, thereby completing the maturation of the autophagosome. After maturation, the autophagosomes fuse with lysosomes to form autolysosomes. This fusion is mediated by a set of soluble NSF attachment protein receptor proteins, including STX17, SNAP29, and VAMP8, in association with ATG14L.[89–91] During this process, the ATG conjugation system promotes fission of the autophagosomal membranes, resulting in degradation of the inner membrane of the autolysosome. The LC3 on the outer autophagosomal membrane is recycled to generate new autophagosomes, while acid hydrolases in the lysosomes degrade the cargos. Although autophagy degrades cellular materials in a non-selective manner, it can degrade specific targets with the aid of autophagy receptor proteins that connect polyubiquitinated cargos and autophagosomes. For example, p62/SQSTM1 (p62) can interact with both polyubiquitinated proteins and LC3.[92] Depolarized mitochondria recruit Parkin, an E3 ligase, through a Pink1-dependent mechanism, thereby inducing K63-linked polyubiquitination of outer mitochondrial membrane proteins. In this way, damaged mitochondria interact with p62 and are sequestrated by autophagosomes via the interaction between p62 and LC3. NBR1, NDP52, and optineurin also act as ubiquitin-binding LC3 receptors.[93] Alternatively, damaged mitochondria up-regulate proteins with an LC3-interacting domain, termed cargo-localizing LC3 receptors, including Bnip3, Nix, Bcl2-L-13, and FUNDC1, allowing sequestration of damaged mitochondria by autophagosomes (reviewed in reference 93). As we discuss below, the

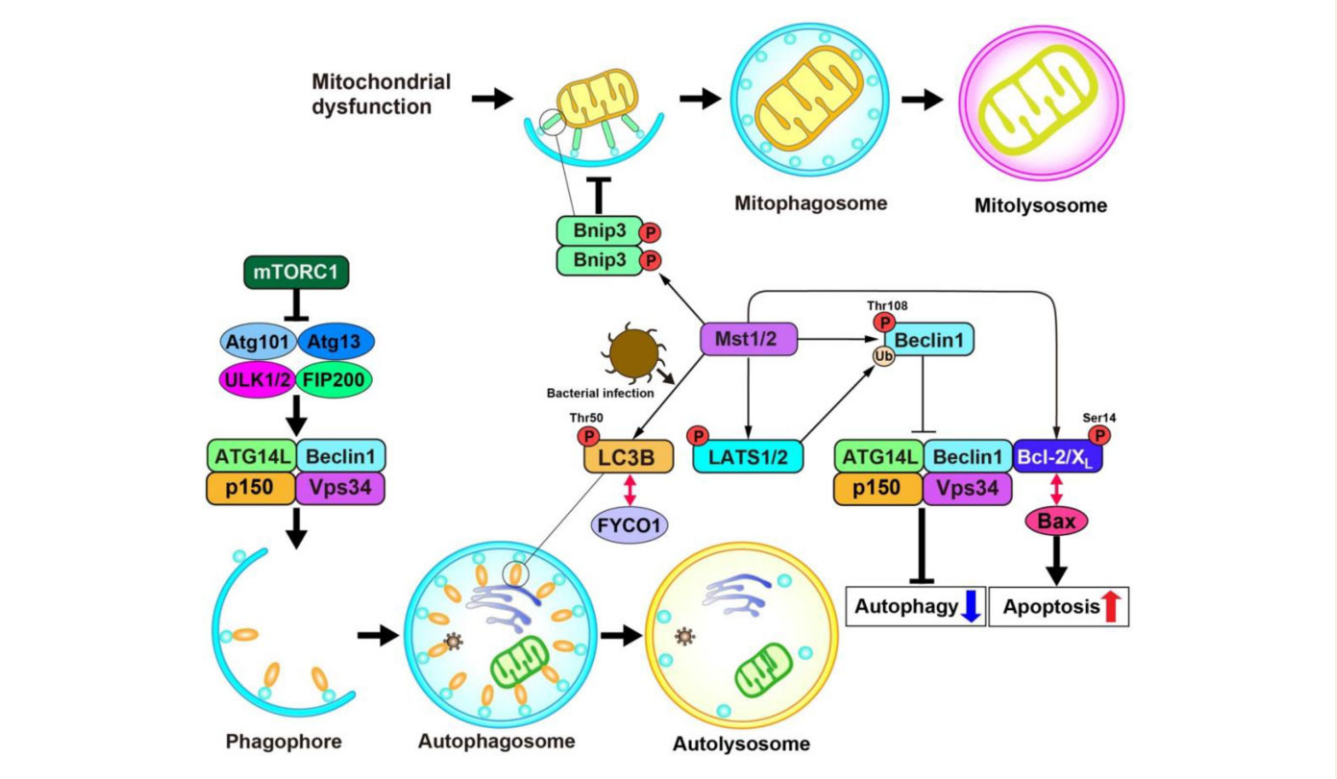

Figure 3 A schematic representation of the roles of Mst1/2 in the regulation of the autophagy machinery. Mst1/2, major components of the Hippo pathway, negatively regulate autophagy via direct phosphorylation of substrates that do not belong to the authentic Hippo pathway (see text for details and definitions of abbreviations). Please note that Mst1/2 stimulate autophagy by phosphorylating LC3B in *C. elegans* and mouse fibroblasts. It is likely that the suppressive effect of Mst1 upon Beclin 1 is predominant in cardiomyocytes. Bax, Bcl-2-associated X protein; Bcl-2/X_L, B-cell lymphoma 2 and X_L; Bnip3, Bcl-2 interacting protein 3; FYCO1, FYVE and coiled-coil domain autophagy adaptor 1.

Hippo pathway interacts with the mechanisms of autophagy and mitophagy at multiple steps, thereby affecting various cellular function in conjunction with its effects upon cell growth and death.

4. The role of the Hippo pathway in modulating autophagy

Growing evidence suggests that the Hippo pathway is intimately involved in autophagy, thereby affecting the survival and death of cardiomyocytes.[65,94] Interestingly, autophagy can also affect the activity of the Hippo pathway, either directly through degradation of the components of the Hippo pathway or secondarily through stimulation or suppression of cell growth and death.[10,12] Here, we introduce examples of the interaction between the Hippo pathway and autophagy, with an emphasis on their effect upon the heart and the cardiomyocytes therein.

4.1 Mst1 is a potent inhibitor of autophagy in cardiomyocytes

Although the upstream components of the Hippo pathway, including Mst1/2 and LATS1/2, are frequently characterized as negative regulators of YAP/TAZ, their functions can also be mediated independently of YAP/TAZ via direct phosphorylation of substrates that do not belong to the authentic Hippo pathway (*Figure 3*). One such example is Mst1-induced phosphorylation of Beclin 1, which potently inhibits autophagy in cardiomyocytes.[13] Mst1 is activated in the heart during the chronic phase of post-MI cardiac remodelling and inhibits autophagy through phosphorylation of Beclin 1 at threonine (Thr) 108, located in the BH3 domain. Phosphorylation of Beclin 1 Thr108 enhances the binding of Bcl-2, a negative regulator of Beclin 1, through electrostatic interaction. Binding of Bcl-2 to Beclin 1 inhibits the PI3 kinase activity of the Atg14L-Beclin 1-Vps34 complex, which in turn inhibits autophagy. The strong inhibitory effect of Mst1 on autophagy is not mimicked by down-regulation of YAP, indicating that the effect of Mst1 is not mediated through the canonical Hippo pathway.[13] A recent study using protein–peptide binding assays and X-ray crystal structural analyses confirmed that Thr108 phosphorylation modestly increases the affinity of a peptide corresponding to the Beclin 1 BH3 domain for Bcl-2/Bcl-xL.[13] Since the study was conducted using a truncated protein and peptides, the nature of the interaction between Beclin 1 and Bcl-2/Bcl-xL could have been better evaluated under conditions that preserve the physiological interactions of membrane proteins, such as cryo-electron microscopy analyses. Interestingly, the Beclin 1 BH3 domain competes with the Bax BH3 domain for Bcl-2/Bcl-xL binding: Mst1-mediated Beclin 1– Bcl-2/Bcl-xL interaction promotes dissociation of Bcl-2/Bcl-xL from Bax[13] in cardiomyocytes. Thus,

activation of Mst1 not only inhibits autophagy through Beclin 1-Bcl2/Bcl-xL interaction, but also stimulates apoptosis through Bax activation, both of which could facilitate cardiomyocyte death during stress conditions, such as heart failure. Activated Mst1 also phosphorylates Bcl-xL at Ser14 in the BH4 domain, thereby abrogating Bcl-xL-Bax binding in the heart,[51] which may assist Bcl-xL binding to Beclin 1. Thus, Mst1 acts as a switch, facilitating a shift from autophagy to apoptosis by phosphorylating Beclin 1 and Bcl-xL.

In addition to suppressing autophagy via mTOR activation, a well-established mechanism of autophagy inhibition,[95] we believe that activation of Mst1 is itself a major mechanism mediating the suppression of autophagy in the heart in response to stress.[83] This view has been supported by others using mouse models of myocardial injury and cardiomyopathy.[96–98] We have shown that down-regulation of autophagy below physiological levels plays an important role in mediating the progression of heart failure.[13] Currently, the molecular mechanism through which autophagy is down-regulated during the chronic phase of heart failure is not fully understood. Mst1 is time-dependently activated in cardiomyocytes in response to pressure overload in the mouse heart.[45] In addition, Mst1 is also activated in other cell types, including macrophages and endothelial cells, in the heart, and suppression of autophagy in non-myocytes may also contribute to the development of inflammation and cardiac dysfunction.[99,100] Thus, we propose that Mst1-induced suppression of autophagy and stimulation of apoptosis both contribute to the progression of heart failure.

Mst1 also inhibits mitophagy in the heart.[97,101,102] Since Mst1 is translocated to mitochondria during stress,[51] it is likely that Mst1 inhibits autophagosome formation for mitophagy as well. Mitophagy mediated by conventional mechanisms of autophagy is activated transiently in the mouse heart in response to high fat diet consumption. Interestingly, Mst1 is activated when conventional mitophagy is inactivated during the acute phase of high fat diet consumption.[46] Mitophagy is also inhibited by endogenous Mst1 in the heart during sepsis.[103] In adipocytes, STK3/Mst2 and STK4/Mst1 increase mitophagy by regulating the phosphorylation and dimerization status of the mitophagy receptor Bnip3.[104] Inactivation of STK3/Mst2 and STK4/Mst1 increases mitochondrial mass and function, promotes browning of the adipose tissue, and confers resistance to metabolic dysfunction. Thus, the stimulation of mitophagy by STK3/Mst2 and STK4/Mst1 appears to be detrimental in adipocytes. Further investigation is required to clarify the role of STK4/Mst1 in mediating mitophagy in the heart and its functional significance.

It should be noted that the effect of Mst1 upon autophagy appears to be stimulus specific. During autophagy activation in response to bacterial infection, STK3/Mst2 and STK4/Mst1 phosphorylate LC3B at Thr50, thereby stimulating autophagy in mouse fibroblasts and *Caenorhabditis elegans*.[105] A follow-up study conducted in the same laboratory showed that LC3B phosphorylation lowers LC3B binding to the transporter protein FYCO1, which in turn induces retrograde transport of autophagosomes within cells, thereby leading to lysosomal degradation.[106] On the other hand, a recent study showed that FYCO1 positively regulates autophagic flux in cardiomyocytes.[107] Thus, the role of FYCO1 in regulating autophagy appears to be cell-type-dependent (*Figure 2*). The molecular mechanisms through which different stresses direct STK4/Mst1 to particular targets in the autophagy machinery remain to be elucidated. Furthermore, the distinct effects of Mst1 upon autophagy in cardiomyocytes and fibroblasts may indicate the existence of unique mechanisms of autophagy in cardiomyocytes. Further investigation is needed to elucidate these mechanisms.

Other upstream components of the Hippo pathway, including LATS1, LATS2, STK38/NDR1, Mst4, and Rassf1A, also negatively and positively

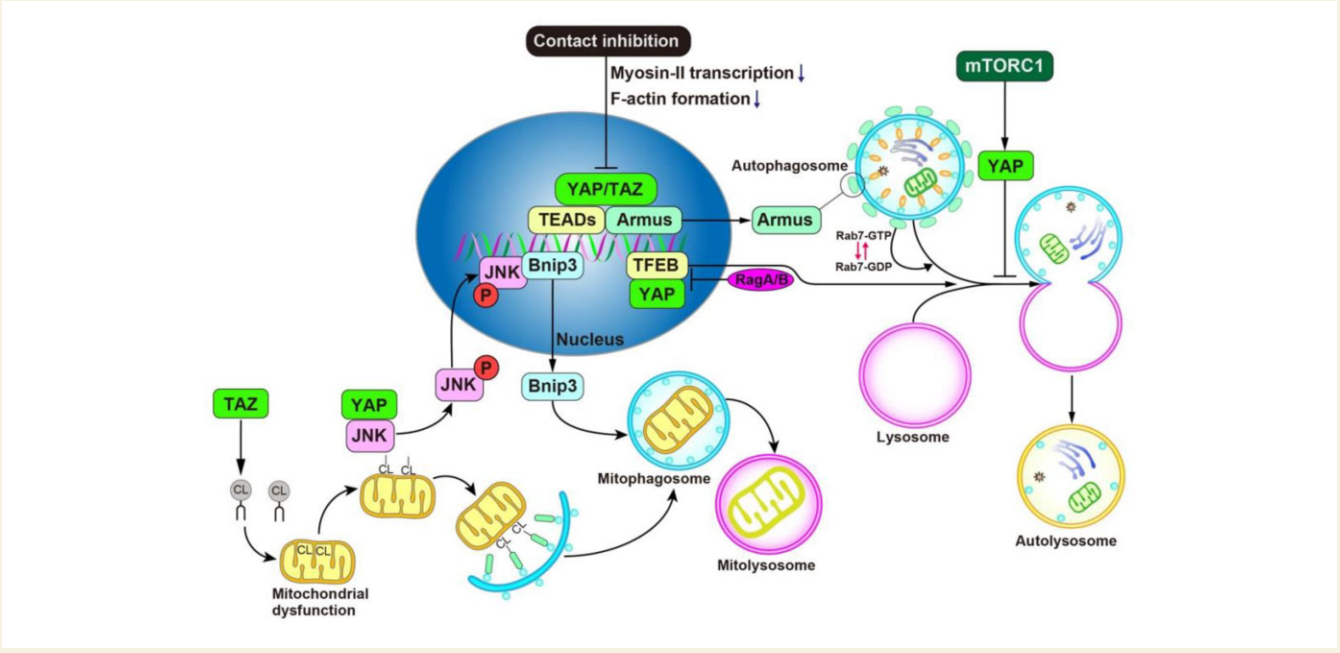

Figure 4 A schematic representation of the roles of YAP/TAZ in the regulation of the autophagy machinery. YAP/TAZ critically regulates autophagy in a manner dependent and/or independent of transcription, which in turn modulates the intracellular environment (see text for details and definitions of abbreviations). CL, cardiolipin; JNK, c-Jun N-terminal kinase; RagA/B, Ras-related GTP-binding protein A and B; TFEB, transcription factor EB.

affect autophagy in non-cardiac cells.[108–110] The mechanisms of action are diverse, acting as kinases or scaffolds, and the overall influence upon autophagy appears context dependent. The role of these mechanisms in the regulation of autophagy and their functional significance in the heart and the cardiomyocytes therein are poorly understood.

4.2 The role of YAP in regulating autophagy

Studies conducted in non-cardiac cells have shown that YAP transcriptionally regulates autophagy, which in turn regulates cell plasticity, survival, and proliferation.[111,112] Since these important properties are required for tissue regeneration and cancer growth, it has been suggested that there is an intimate interaction between YAP and autophagy (*Figure 4*). In cancer cells, YAP/TAZ promote the fusion of autophagosomes and lysosomes by up-regulating Armus, a Rab7-GAP required for autophagosome turnover, thereby sustaining the tumour cell phenotype.[112] YAP/TAZ inactivation during contact inhibition attenuates autophagy by suppressing the transcription of myosin-II genes and the formation of F-actin stress fibres, which in turn inhibits survival and proliferation in non-cancerous cells.[111] On the other hand, YAP inhibits autophagic flux in human umbilical vein endothelial cells and isolated vascular tissues, thereby promoting cellular senescence, possibly through an mTOR-dependent mechanism.[113]

YAP promotes mitophagy through a c-Jun N-terminal kinase (JNK)-dependent mechanism in cancer cells.[112] YAP physically interacts with and induces phosphorylation of JNK, thereby facilitating nuclear

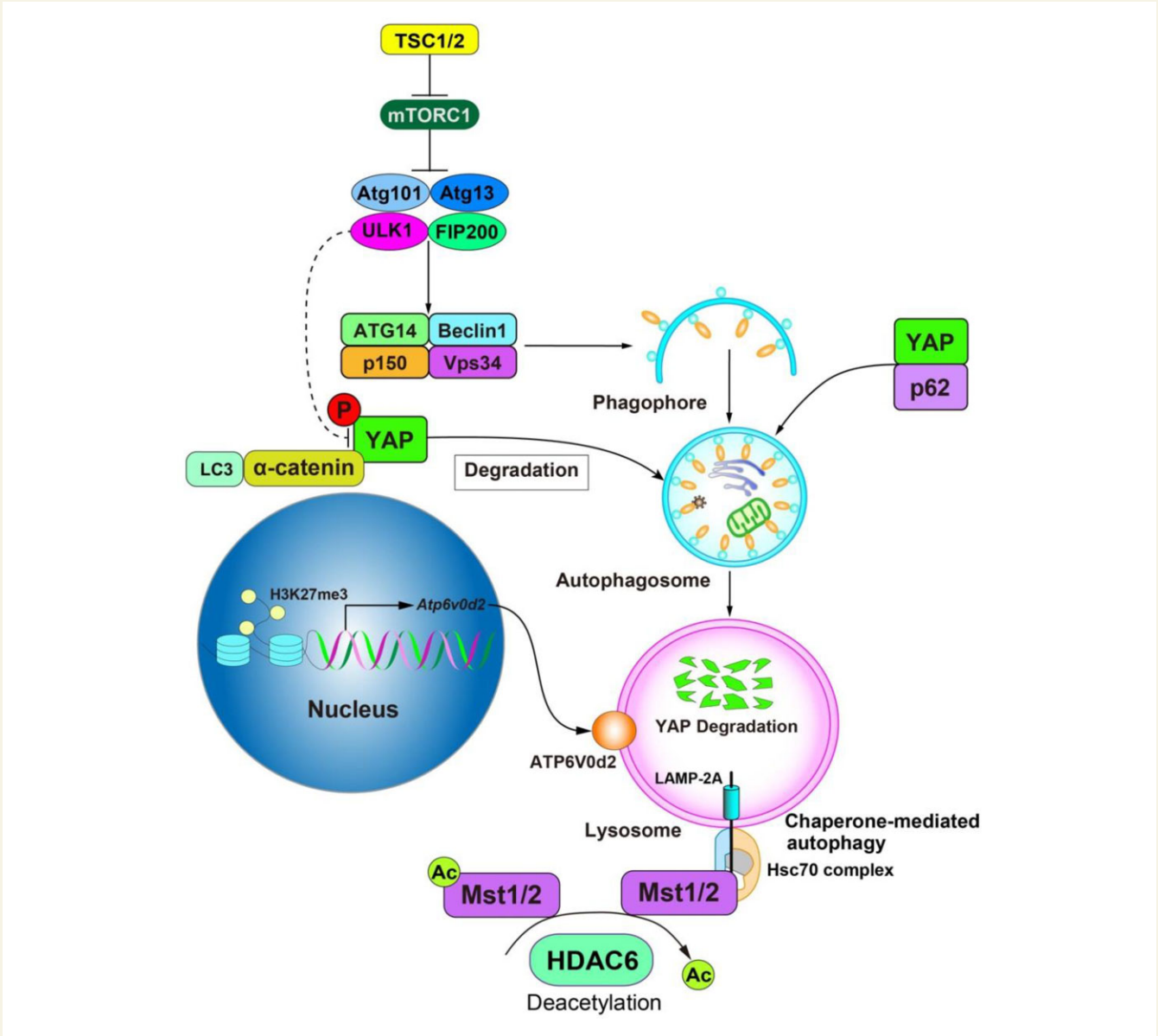

Figure 5 A schematic representation of autophagy-mediated regulation of YAP. The level of YAP is critically regulated by the autophagy machinery via various pathways (see text for details and definitions of abbreviations). Ac, acetylation; ATP6V0d2, ATPase H^+ transporting V0 subunit D2; H3K27me3, histone H3 trimethyl Lys27; HDAC6, histone deacetylase 6; Hsc70, heat shock cognate 71 kDa protein; LAMP-2A, lysosome-associated membrane glycoprotein 2 A; TSC1/2, tuberous sclerosis complex 1 and 2.

localization of JNK, which, in turn, enhances transcriptional up-regulation of Bnip3, a critical regulator of mitophagy.[114]

We recently demonstrated that YAP promotes autophagosome formation in the heart in a mouse model of LSD.[12] LSD is caused by impairment of lysosomal degradation and characterized by accumulation of substrates, such as glycogen, that are normally degraded in lysosomes. Since lysosomal function is essential for cargo degradation by autophagy, autophagic flux is often impaired as well. In cardiac-specific RagA/B knockout (RagA/B cKO) mice, a mouse model of LSD,[12] lysosome acidification is impaired and cardiomyopathy develops. Although autophagic flux is permanently blocked in this mouse model, autophagosome formation is stimulated, most likely due to a cellular attempt to restore autophagy, which in turn causes a marked accumulation of autophagosomes in the heart. Excessive accumulation of autophagosomes has been shown to be detrimental in several mouse models of LSD, including RagA/B cKO mice.[115] What is the underlying mechanism mediating the excessive accumulation of autophagosomes? As we discuss in the next section, YAP accumulates when autophagy is inhibited and, in RagA/B cKO mice, YAP interacts with TFEB, a master regulator of lysosome biogenesis and autophagy, in the nucleus, stimulating transcription of genes involved in autophagosome formation. Down-regulation of YAP or direct suppression of autophagosome formation through down-regulation of ATG7 attenuates the development of cardiomyopathy in RagA/B cKO mice.[12] Although YAP-TFEB is activated to compensate for the impaired autophagic flux, because of the presence of a permanent block at the lysosomal level, this activation only exacerbates accumulation of autophagosomes without improving autophagic flux. In breast cancer cells, YAP promotes autophagic flux in response to nutrient deprivation by stimulating autolysosome degradation through a TEAD-dependent mechanism.[116] Whether TFEB is also involved in this process remains to be determined.

It has been shown that stimulation of endogenous YAP in the heart is salutary during cardiac remodelling after MI.[25,57,58,64,65] Although stimulation of myocyte proliferation appears to be the major mechanism mediating this effect, YAP also improves mitochondrial quality by up-regulating Parkin, an E3 ubiquitin ligase.[65] Elucidating the detailed molecular mechanism through which YAP up-regulates Parkin may lead to development of a novel intervention by which mitochondrial quality may be improved during cardiac remodelling.

4.3 Autophagy regulates the level of YAP

Although macroautophagy was originally described as a non-selective mechanism of cellular degradation, increasing lines of evidence suggest that autophagy can target specific proteins and organelles for lysosomal degradation through interaction between autophagy receptors possessing the LC3-interaction sequence and their targets (*Figure 5*).[117] YAP is accumulated in TSC1/TSC2-deficient human perivascular epithelioid cells, due to mTOR-mediated suppression of autophagy.[11] The cross talk between mTOR and YAP, master regulators of nutrient sensing and cell growth, respectively, strongly predisposes cells to conditions favouring tumourigenesis. Autophagic degradation of YAP has also been reported in other organs and cells[101] and has been established as an important mechanism controlling the level of YAP in many cell types. Transcription of ATP6V0d2, a lysosome V-ATPase that mediates YAP degradation in lysosomes, is negatively regulated by H3K27me3, an epigenetic mechanism. Ser metabolism negatively regulates expression of ATP6V0d2 through H3K27me3. Thus, Ser metabolism negatively regulates lysosomal degradation of YAP and induces YAP accumulation.[118] YAP/TAZ activity is regulated by autophagy-mediated degradation of α-catenins, LC3-interacting proteins. The directionality of YAP/TAZ regulation through this mechanism critically depends upon the level of α-catenin; whereas high levels of α-catenin allow autophagy to positively regulate YAP/TAZ, low α-catenin causes YAP/TAZ to be activated when autophagy is inhibited.[119] ATG1 phosphorylates Yorkie, a homologue of YAP, thereby suppressing its binding to Scalloped and transcription of downstream targets in *Drosophila*. Overexpression of ATG1 inhibits Yorkie independently of Hippo-Warts signalling, resulting in suppression of overgrowth and proliferation of the epithelium.[120] Induction of autophagy in response to disturbed shear stress negatively affects the nuclear import and transcriptional activation of YAP in endothelial cells through unknown mechanisms.[121] Taken together, these studies demonstrate that autophagy regulates the activity of the Hippo pathway through multiple mechanisms.

We have shown recently that YAP accumulates in the heart when autophagy is suppressed, as in LSDs.[12] Since YAP physically interacts with p62, a LC3-receptor protein, YAP can be sequestrated by autophagosomes through p62, an autophagy receptor. Suppression of the baseline level of autophagy increases the protein level of YAP in cardiomyocytes; thus, the basal level of YAP in cardiomyocytes is critically regulated by selective autophagy. YAP can, in turn, regulate transcription of downstream targets when autophagy is suppressed. Since autophagy is down-regulated in many pathologically relevant conditions, including heart failure, diabetes, and ageing, it would be interesting to investigate how autophagy down-regulation in these conditions affects YAP and YAP-mediated transcription in the heart. On the other hand, an excessive increase in autophagic flux could reduce the level of YAP, which in turn may contribute to cardiomyocyte death. It would be of great interest to determine whether supplementation of YAP alleviates cell death in the presence of excessive activation of autophagy.

The cross talk between autophagy and the Hippo pathway can also be observed at the level of Mst1. In breast cancer cells, HDAC6-induced deacetylation of Mst1 promotes degradation of Mst1 through chaperone-mediated autophagy, thereby contributing to tumourigenesis.[122]

5 Concluding remarks

In summary, we have discussed the essential role(s) of the Hippo pathway in regulating autophagy to maintain cellular homeostasis in the heart. The Hippo pathway and autophagy affect each other's activities; thus, the Hippo pathway serves as either a positive or a negative feedback mechanism to regulate the activity of autophagy. In addition, both Mst1 and YAP act as critical regulators of cell death and survival by affecting both apoptosis and autophagy in cardiomyocytes. It is, therefore, important to understand the consequences of the cross talk between apoptosis and autophagy mediated through Mst1 and YAP. Since autophagy has both adaptive and maladaptive functions in the heart,[52] autophagic activity must be maintained at appropriate levels. Activation of Mst1 suppresses autophagy below physiological levels, whereas that of YAP can induce excessive accumulation of autophagosomes in the presence of lysosomal dysfunction. Thus, both Mst1 and YAP may represent useful targets of therapeutic intervention in the presence of autophagy dysregulation in the heart. In particular, accumulating evidence suggests that activation of Mst1 is involved in suppression of autophagy in various heart disease conditions. To translate the findings obtained from experimental animals to the clinic, it would be important to investigate how autophagy is regulated in the human heart under various stress conditions. Currently, the

assessment of autophagy in patients is technically challenging. Thus, more investigation is warranted to elucidate the role of autophagy in various disease conditions and the involvement of the Hippo pathway therein.

Authors' contributions

Y.M., D.Z., and J.S. wrote the manuscript, and the authors reviewed and approved the manuscript for publication. J.N. participated in the research project on the Hippo pathway–autophagy interaction in the Sadoshima laboratory and conducted a critical reading of the manuscript.

Conflict of interest: none declared.

Funding

This work was supported in part by U.S. Public Health Service Grants HL67724, HL91469, HL102738, HL112330, HL138720, HL144626, HL150881, and AG23039 (to J.S.), the American Heart Association Merit Award 20 Merit 35120374 (to J.S.), and by the Fondation Leducq Transatlantic Network of Excellence 15CVD04 (to J.S.).

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